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SIZE DIMORPHISM IN ADULT SPERMATOZOA OF ANASA TRISTIS.[1]

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I. Introduction.

The work on which this paper is based was undertaken with the object of determining the existence or non-existence of size dimorphism in the mature spermatozoa of *Anasa tristis*. Spermatogenesis studies of this form have shown that one half of the spermatozoa receive one more chromosome each than the other half. A corresponding difference in size of the mature spermatozoa may therefore be expected.

The study was carried on during the academic year 1912–1913, under the direction of Professor Charles Zeleny, to whom my thanks are due for his many valuable suggestions.

II. Materials and Methods.

1. *General Account.*—The material used in this study was collected from two localities, Urbana, Ill., and Bradford, N. H., during the month of October, 1912. The testes of these individuals were teased out in normal salt solution and made up into temporary and permanent smear preparations.

Smears of adult spermatozoa were prepared by the aceto-carmine method and by the osmic-hæmatoxylin method. In addition *intra vitam* staining was tried, but without success. The first mounts were made up as aceto-carmine preparations. Although this affords an excellent preparation for the study of spermatogenesis, it is not a good method to use in the study of adult spermatozoa. Curling is frequent. Distortion by swelling

[1] Contributions from the Zoölogical Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 23.

occurs more than is desirable. In the head of the spermatozoön no *inner chromatic rod* is differentiated from the cytoplasmic envelope. Moreover, it often takes several days to measure a considerable number of individuals, so that temporary preparations, such as those obtained with aceto-carmine, are objectionable. For this same reason *intra vitam* staining was abandoned.

For the most of the preparations Delafield's hæmatoxylin was used and proved much more successful than Schneider's aceto-carmine. The testis was taken out of the individual and placed in normal salt solution. It was then transferred to a slide that had been previously coated with a thin film of albumen fixative. The spermatozoa were teased out on the slide and examined in a drop of some fluid, such as Ringer's, to determine their motility or non-motility. After most of the solution had been allowed to evaporate so that only a bit of moisture was left around the spermatozoa, the preparation was fixed in osmic fumes for thirty seconds and then stained in Delafield's hæmatoxylin. This method was found to give a maximum number of straight spermatozoa with an *inner chromatic rod* well differentiated.

The microscope used in the measurements was equipped with a Leitz 2 mm. oil immersion objective and Zeiss compensating oculars. A large number of individuals was measured and a curve of variability plotted. In every individual it was the *length of the inner chromatic rod* which was determined. A description of this chromatic rod occurs later under the description of a mature spermatozoön.

2. *Sources of Error.*—The probable sources of error that need special consideration are (1) imperfect technique in preparations; (2) immaturity of the spermatozoa; or (3) incorrect measurement.

(1) *Error Due to Imperfect Technique in Preparations.*—Several smears of mature spermatozoa were prepared by the aceto-carmine method. Measurements made from them were not uniform, due to faulty technique. Many of the sperm heads had been curled or shrunken in fixation. Lack of an exact tip or base of the chromatic rod could have given rise to an error of from 1μ to 5μ in measurements of length. Distention and distortion were common. On account of the faulty prepara-

tions, the results obtained from them have not been included in the data.

In the aceto-carmine preparations it was found that drawing off the excess stain by a bit of filter paper as recommended, carried along with it many spermatozoa. In fact, preliminary measurements of such preparations showed a prevalence of larger spermatozoa on the side toward which the drawing was being made. Precautions were taken to eliminate this fault by fixing the spermatozoa to the slide with a thin film of albumen fixative. Also osmic fumes were used in preference to a liquid killing agent and Delafield's hæmatoxylin, instead of some more complicated stain, in order that all the spermatozoa might be preserved. This osmic-hæmatoxylin combination has been found particularly effective in producing straight spermatozoa with well-marked chromatic elements. In the preparations from which data were compiled from ninety-five to ninety-seven per cent. of the spermatozoa were sufficiently straight for measurement.

(2) *Error Due to Immaturity.*—When preliminary mounts were made about the first of October, 1912, the material was found to be too immature for the purpose of this work. All stages in spermatogenesis were present from the spermatogonium to the young spermatids, but no adult spermatozoa were found.

One of the problems confronting the writer in determining suitable preparations for study was the recognition of a truly mature spermatozoön. In general, the attenuated character of the head, with the chromatic rod (nuclear element) extending almost its entire length, is sufficient for the recognition of a mature spermatozoön. On that basis all preparations where more than five per cent. of the individuals were immature were discarded. In order to satisfy himself more fully of the maturity of the individuals measured, the author examined all of them in Ringer's solution, which demonstrated their motility. In this fluid the developing spermatids and immature spermatozoa were quiet, while the fully ripe spermatozoa were active for a considerable length of time. However, motility is not to be taken as a general criterion of maturity. In the house-fly, *Musca domestica*, for example, immature forms are motile. Again, these

spermatozoa were similar in all respects to those obtained at Woods Hole in August, 1913, from adult males of this species. Because of their structure and specific reaction the author is assured that all spermatozoa entering into consideration in the data are mature individuals.

The description of a mature spermatozoön becomes necessary at this point. As far as the writer has been able to discover no one has described a *chromatic rod* with cytoplasmic sheath for the mature spermatozoön of *Anasa tristis*. Paulmier's description and figures show no such structure. Meek's figure for the "penultimate stage" of maturing spermatozoa of *Stenobothrus viridulus* shows something of the nature of this chromatic rod (Plate III., Fig. 36).

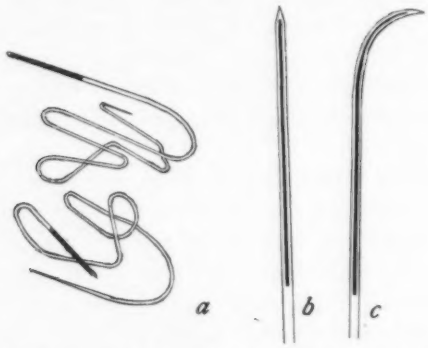

FIG. 1. Mature spermatozoa. (*a*) Entire spermatozoa; (*b*) straight sperm-head, the inner chromatic rod of this type was measured; (*c*) curved sperm-head, a characteristic type not measured.

The author's preparations agree with Paulmier's description of the mature spermatozoön of *Anasa tristis* in having no middle piece distinguishable, so that the tail connects directly with the posterior end of the head. The head has the chromatic elements compacted into a long attenuated rod of nearly constant diameter throughout its entire length. This *chromatic rod* is enveloped by a cytoplasmic capsule. The latter is also attenuated, and has a constant outer diameter. The length of the chromatic rod ranges from 24μ to 36μ and the width from $\frac{1}{3}\,\mu$ to $\frac{2}{3}\,\mu$. Although the width is too minute for practical measurement, in general, it seems to be proportional to the length. The cytoplasmic

sheath is two to three times as thick as the chromatic rod. It extends 2μ to 3μ anterior to the anterior end of the chromatic rod, and is continuous posteriorly with the sheath of the axial filament. Considerable observation has shown that the frequency curve for chromatic rod length does not differ from the

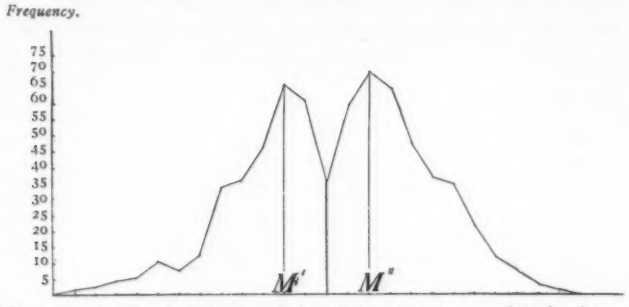

FIG. 2. Bimodal curve of variability of chromatic-rod length for 11. Greater number of spermatozoa grouped around the upper mode, M''.

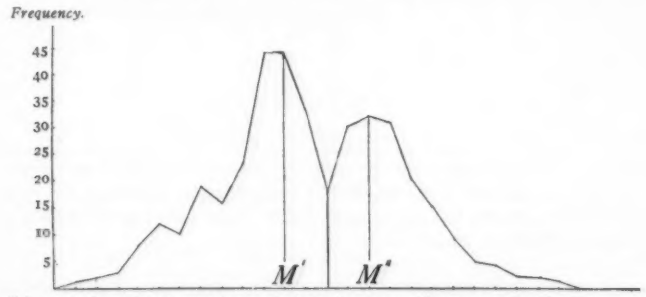

FIG. 3. Bimodal curve of variability of chromatic-rod length for 11. Greater number of spermatozoa grouped around the lower mode, M'.

curve for the entire head. Unlike the adult spermatozoa described by Paulmier ('99) and Stevens ('05), the author's preparations show a *distinct region of cytoplasm around the chromatic rod*, and fail to demonstrate that the sheath is entirely, or even mostly "used as food material for the growth of the tail sheath," as Paulmier described it (p. 254; also Figs. 56 and 57). The author's hæmatoxylin preparations show no acrosome or

centrosome such as Paulmier described for this form. This difference might be claimed to exist because of the different staining capacities of Delafield's hæmatoxylin, used in the author's preparations, and iron-alum hæmatoxylin which Paulmier employed. However, Meek's preparations of the "ante-penultimate stage" of *Stenobothrus*, which were stained with the iron-alum hæmatoxylin, show a distinct chromatic rod and sheath, but no acrosome and a diminishing centrosome.

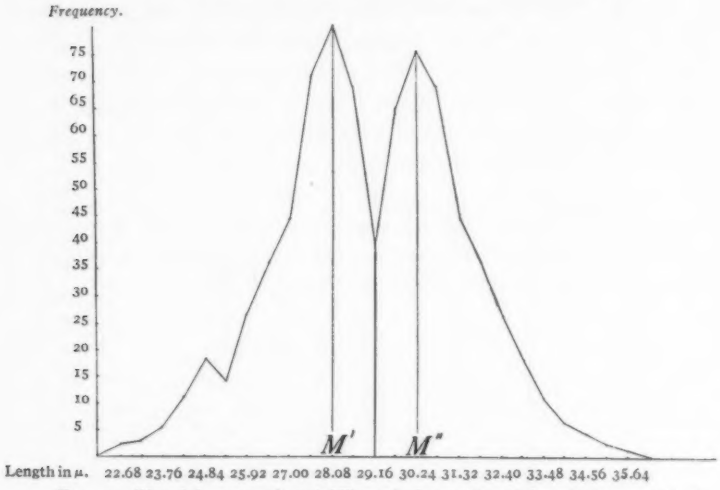

FIG. 4. Bimodal curve of variability of chromatic-rod length for 11 and 11 combined. Number of spermatozoa around both modes approximately equal. Almost bilaterally symmetrical.

Certain spermatozoa of unusually large size were observed. These are the so-called "giant spermatozoa." Such "giant spermatozoa" have been described by Henking ('91), and Wilcox ('95) for various species, and by Paulmier ('99) for *Anasa tristis*. Paulmier described those of "double and quadruple" the normal size, and attributed the phenomena "to the non-completion of one or both of the spermatocyte divisions." The author has observed only the double-sized forms. They are found in about one per cent. of the measurements. These spermatozoa are about one and one fourth times the length of the average normal spermatozoön, and contain about twice the amount of chro-

matic material of the normal forms. Smith's "giant spermatozoa" of hybrid pigeons, which are twice the normal length, do not appear to belong to this category. In a few cases the author has observed two mature chromatic rods of normal length within a single cytoplasmic membrane. Evidently these were cases where the chromatic elements had divided normally, but

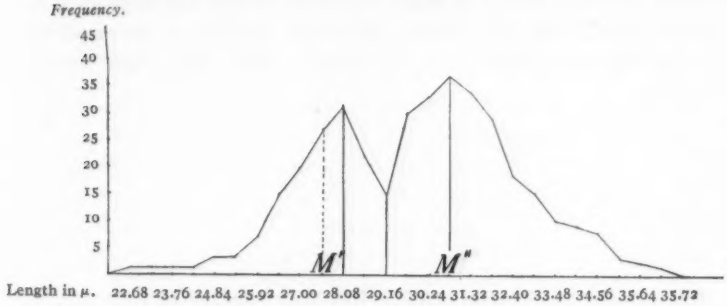

FIG. 5. Bimodal curve of variability of chromatic-rod length for 2r. Greater number of spermatozoa around the upper mode, M".

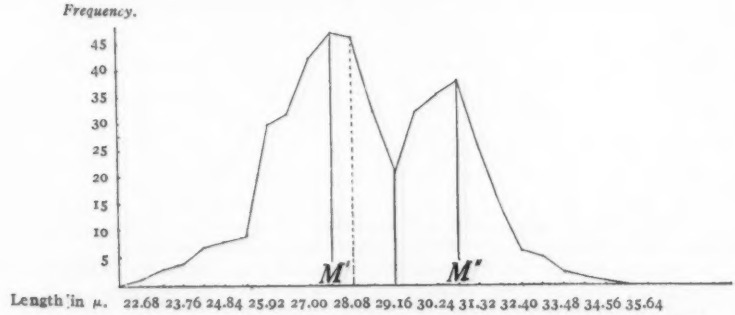

FIG. 6. Bimodal curve of variability of chromatic-rod length for 2l. Greater number of spermatozoa around the lower mode, M'.

where the cytoplasm had failed to divide, so that the two chromatic elements had developed together side by side within the same sheath. It is interesting to note that such "twin" spermatozoa are always of different lengths, varying from 1.5μ to 3μ from each other. "Giant spermatozoa" of this same type have been observed by the author in smear preparations of the housefly, *Musca domestica*.

The "giant spermatozoa" from the author's preparations had no definite range of variability but blended into the upper reaches of the curve for the normal spermatozoa. The most extreme "giant forms," as well as those that are less extreme, might be considered as wide variants in the frequency distribution of normal forms. If the average normal length is taken, and a "giant spermatozoön" of twice that chromatic volume is computed, then such a form would still fall within the upper reaches of the normal frequency distribution. It is evident, then, that such forms

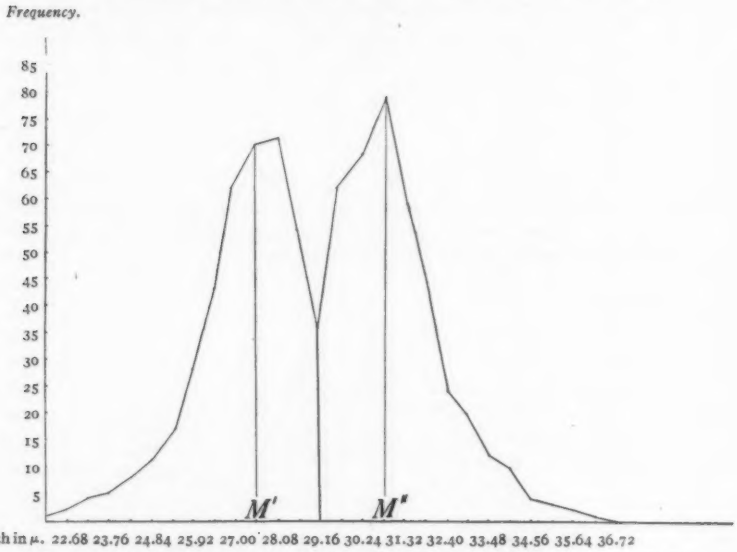

FIG. 7. Bimodal curve of variability of chromatic-rod length for 2r and 2l combined. Number of spermatozoa around both modes approximately equal. Almost bilaterally symmetrical.

can be considered as "giant spermatozoa" only when an arbitrary upper limit is placed to the normal frequency curve and these wide deviants considered as twice the volume of average spermatozoa.

(3) *Error Due to Incorrect Measurement.*—It is evident that, for the purpose of measurement, the inner chromatic rod must be well differentiated both anteriorly and posteriorly. Aceto-carmine preparation failed in this respect, while the Delafield's

brought it out clearly. Because the posterior limit of the chromatic rod is more easily located than the anterior limit, all measurements were made from the posterior end forward.

Another item to be taken into consideration is that the spermatozoön may not always be in a perfectly horizontal position on the slide, and hence all parts of it may not be in focus at once.

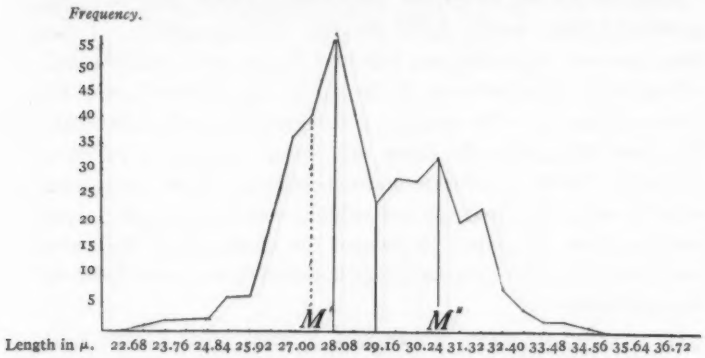

FIG. 8. Bimodal curve of variability of chromatic-rod length for 3r. Greater number of spermatozoa around lower mode, M'.

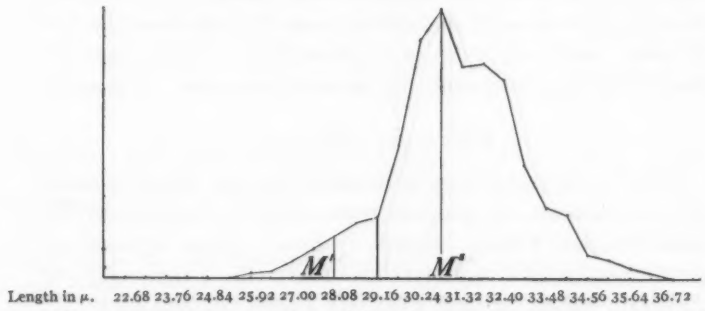

FIG. 9. Unimodal curve of variability of chromatic-rod length for 3l. M'' the only mode.

This may be due to a downward or upward bending or curving of the anterior end of the head, or to a tilting of the entire head, whether curved or straight. To preclude any danger of incorrect measurement all such spermatozoa were recorded separately and only entered in the data with those which were distinctly crooked.

The total of all spermatozoa, curved, crooked and tilted, which were not measured amounted to three to five per cent. of the numbers measured. Had this group been all of a critical length they would not have been sufficient in a single case to change the fundamental character of the curve of variability. An example of such a "curved" sperm-head is shown in Fig. 1, *c*.

A question arose as to how many spermatozoa must be measured before the curve is fairly constant and dependable. It has been observed that with one hundred fifty or two hundred individuals the main features of the curve are outlined; that the curve fluctuates in its details up to three hundred individuals; that four hundred individuals are enough to give a curve of variability which is accurate and dependable. Even a measurement of seven hundred ten individuals, which was made in one case (number 1r), failed to change the character of the curve from that secured for the first four hundred of the seven hundred ten individuals.

In addition to these precautions, several other checks were made in measurement. One preparation, number 1r, was measured three times with three different magnifications; the curves for all three measurements were similar. All measurements were made with the writer's same eye, but were checked by other research students in the laboratory. Finally, only the best of light was used while the measurements were being made.

III. Data Obtained.

The data presented were obtained from eight smear preparations taken from four pairs of testes, fixed and stained by the osmic-Delafield method described above. These preparations are designated as 1 right (1r) and 1 left (1l), 2 right (2r) and 2 left (2l), 3 right (3r) and 3 left (3l), 4 right (4r) and 4 left (4l). Taken as a whole the measurements show very clearly that there is a distinct size dimorphism in the spermatozoa. Individual testes, however, differ considerably from each other in details.

Preparations 1r and 1l.—These spermatozoa when examined in Ringer's solution were very active. From one thousand to two thousand individuals were present on each slide preparation, comprising the total number of spermatozoa from each testis.

With ocular no. 8, seven hundred ten individuals of 1r were measured. This number has been shown to be sufficiently large to plot a reliable frequency curve. Spermatozoa selected were taken as random samples from all parts of the slide. The variability curve is shown in Fig. 2. It shows two distinct modes at the lengths 28.08μ and 30.24μ, with a point of depression midway between at 29.16μ. Not only are the two modes equally distant from the intermediate low point, but the entire curve is

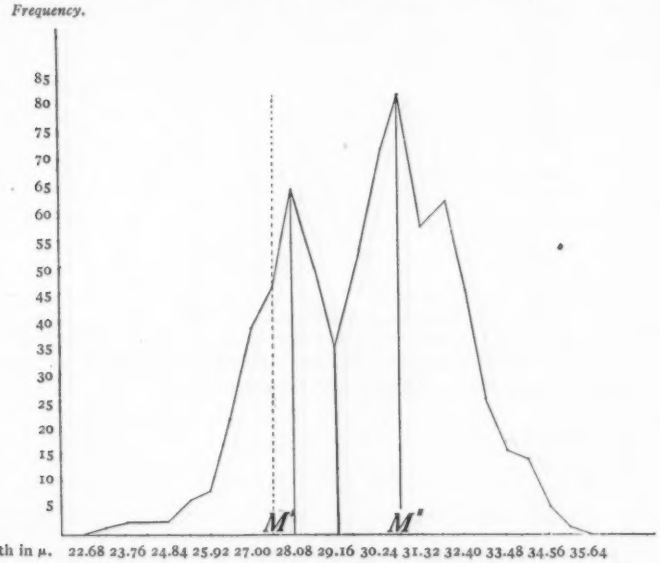

FIG. 10. Bimodal curve of variability of chromatic-rod length for 3r and 3l combined. Number of spermatozoa around upper mode, M'', is greater.

approximately bilaterally symmetrical. This same preparation was measured again with a no. 2 ocular and yet again with a no. 12 ocular. In the use of no. 2 ocular five hundred twenty-four individuals were measured; in the use of no. 12 ocular four hundred twenty-nine measurements were recorded. Both of these give curves agreeing closely to the bimodal curve of the first measurement.

With ocular no. 2, the magnification was hardly sufficient to correctly place the modes. Ocular no. 8 was found most satis-

factory and used in all the following measurements. For all these data each measurement was recorded in sequence and later incorporated into a polygon plot. All crooked or curved individuals were entered separately and not used except as a check.

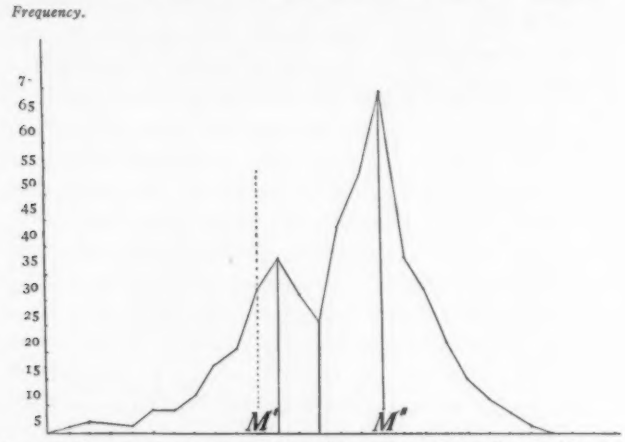

FIG. 11. Bimodal curve of variability of chromatic-rod length for 4r. Greater number of spermatozoa grouped around the upper mode, M''.

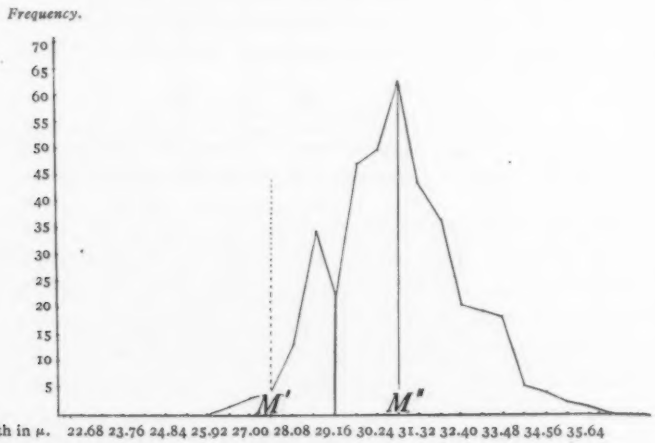

FIG. 12. Bimodal curve of variability of chromatic-rod length for 4l. Greater number of spermatozoa grouped around the upper mode, M''.

They amounted to 5.22 per cent. of the total number measured. Such a per cent. is not sufficient to affect the dimorphic character of the curve, even if it represented spermatozoa all of a critical length, *i. e.*, 29.16μ.

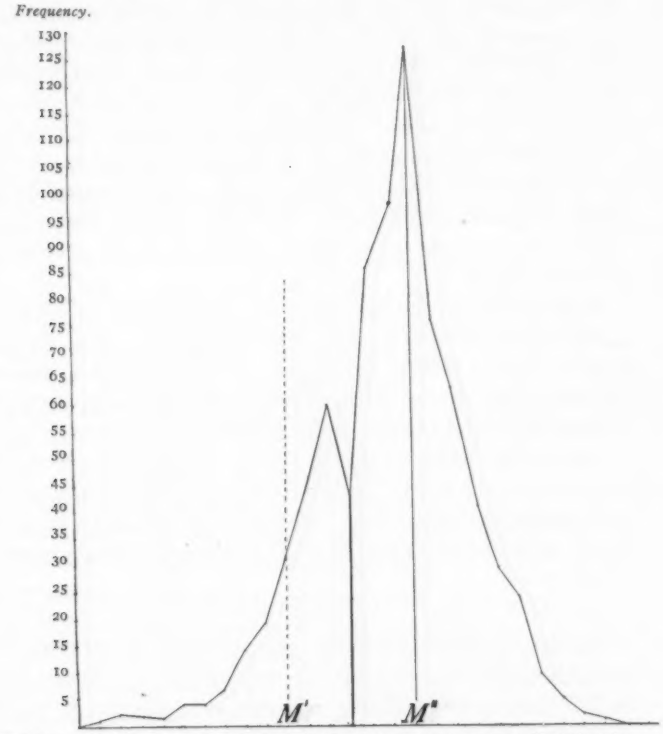

FIG. 13. Bimodal curve of variability of chromatic-rod length for 4r and 4l combined. Number of spermatozoa around upper mode, M″, is greater.

Four hundred individuals from 1l, chosen at random, were measured. This curve also shows a clear dimorphism. It is plotted in Fig. 3.

Preparations 2r and 2l.—Of the right testis 2r, four hundred eighty individuals were measured; of the left testis 2l, four hundred individuals were measured. The plots of these curves, Figs. 5 and 6, show that they are strikingly similar to those of

1r and 1l. It will be noticed, however, that 2r has 30.78μ as a mode of higher frequency, while 2l has 27.54μ as a mode of higher frequency.

Preparations 3r and 3l.—Of 3r only three hundred individuals were measured, because this comprised the total number that was present in the testis. Of 3l five hundred individuals were measured. The curve of 3r is shown in Fig. 8. It is bimodal in character, but with a greater number of individuals grouped around the lower mode. The plot for 3l is shown in Fig. 9. It is unmistakably unimodal in appearance, with the mode at 30.78μ, the length where the upper mode of the preceding bimodal curves occurred. Comparison with the bimodal curves shows plainly that it is the upper half of a bimodal curve, such as that shown in Fig. 2.

Preparations 4r and 4l.—In these preparations four hundred spermatozoa from each testis were measured. 4r is shown in Fig. 11, and 4l in Fig. 12. These are both bimodal and show the dimorphic nature of the spermatozoa, although they differ considerably from the bilaterally symmetrical type.

Preparations made at Woods Hole in August, 1913, from adult males were measured to check the foregoing data. When plotted as a variability curve, they, too, yielded a bimodal curve, with modes at the same lengths as those in the preparations 1r and 1l.

IV. Discussion.

The data presented in the previous section show very distinctly the dimorphic character of the spermatozoa of *Anasa tristis*. On the *basis of the chromatic-rod length* they fall into *two classes*, those with an extra amount of chromatin, and those without such an additional amount. Whatever differences and variations there are among the several curves, the differentiation into two groups remains proved. Such minor deviations as occur are probably due to secondary factors not yet discovered, and do not directly affect the bimodal grouping.

This pronounced dimorphic character of the spermatozoa of *Anasa tristis* must bear some fundamental relation to the dimorphism of chromosome number demonstrated by spermatogenesis studies. It is altogether probable that the mature

spermatozoa grouped around the higher mode of the curve of variability are those possessing the accessory chromosome, and that those grouped around the lower mode lack this accessory chromosome. The approximate equality of the two groups (see Fig. 2), moreover, strengthens the view that the two groups are produced as spermatids in equal numbers.

However, some of the curves do not show this approximate equality, but have marked digressions from a perfectly bilateral symmetry. They may have been produced as spermatids in equal numbers, and the departure from a bilateral grouping may be due to a difference in nourishment; or this deviation may be due to a slightly earlier ripening of the one group and their ejection from the testis.

Another interesting point brought out by the data is the relation between the right and left testis of the same individual. If the curves of 1r and 1l, 2r and 2l, are examined carefully, they are seen to approach bilateral symmetry even more closely when right and left testes of the same individual are plotted together than when each is plotted separately. Such combinations are produced in Figs. 4 and 7. The former is the combination curve for 1r and 1l; the latter is the combination curve for 2r and 2l. In the combination 1r and 1l, of eight hundred individuals plotted an actual difference of only eight is found between the two sides, or a difference of only one per cent. In the combination 2r and 2l, a difference of only five exists between the two sides of the curve, or a difference of only five eighths of one per cent. On the other hand combination curves for the testes of numbers 3 and 4 show no such close bilaterality. A probable explanation for this discrepancy is offered in the relation between the type of curve and abundance of spermatozoa. In the specimens numbers 1 and 2 there was a great abundance of spermatozoa in each testis. Because four to five hundred individuals were found to be reliable as a basis for a curve of variability, the entire number was not measured, but random samples were taken for measurement. In numbers 3 and 4, on the other hand, a different condition existed. For each of these there were not more than five hundred present in each testis. This abundance or scarcity of spermatozoa may have a very vital connection with the fundamental characters of the curve.

In the cases of most evident bilateral symmetry (in numbers 1r and 1l, 2r and 2l), the preparations were made up early in November, when the spermatozoa were still in abundance. In the cases where the curves do not show this bilateral symmetry (in numbers 3r and 3l, 4r and 4l), such a maximum number of mature individuals was not found. In one testis, at least, in each of these pairs (3r and 4r) the majority of the spermatozoa had been ejected from the testis. This fact was shown by the emptiness of the testis upon first examination. We may therefore provisionally consider the *normal condition* as one in which there is a *maximum number of mature spermatozoa*, and the *bilaterally symmetrical curve of variability* as the *best expression of such a norm*.

It might be assumed that this dimorphism in length is due not to any internal factor which marks out two groups of mature spermatozoa, but represents merely a sudden growth in length whereby the maturing spermatozoa pass over the low intermodal point very rapidly, so that few are "caught" in that place. There are definite reasons why such a theory cannot be accepted. The spermatozoa in the upper and lower reaches of the curve show by their structure that they are equally mature. This is shown not only by their general size and shape, but by their staining reaction which is very different from that of immature spermatozoa. Again, immature forms when they do occur are just as likely to occupy the low intermodal portion of the curve as the mature ones. In fact immature forms have been recorded for such a critical length. Such an objection must be put aside as unjustifiable.

V. Summary.

1. The work presented in this paper has proved the presence of size dimorphism in the adult spermatozoa of *Anasa tristis*. Measurements of the length of the chromatic rod of several hundred spermatozoa from each testis, plotted as a frequency curve, have demonstrated two modes, one between 27.54μ and 28.08μ, and the other between 30.24μ and 30.78μ, and the data offer adequate support for the conclusion that length dimorphism exists.

2. This dimorphism in length of adult spermatozoa probably bears a fundamental relation to the dimorphism in chromosome number revealed by spermatogenesis studies.

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EXPERIMENTS WITH TAPEWORMS.

I. Some Factors Producing Evagination of a Cysticercus.[1]

JOHN W. SCOTT.

During the past winter the opportunity came to me to try a series of experiments upon the bladderworm stage of *Tænia serrata*, one of the common dog tapeworms. The work was made easy because an abundance of material could be secured from the cottontail rabbit in this vicinity. As I have found no record of previous experiments of this kind, it has occurred to me that a brief account of them might be of general interest. One series of experiments in particular I shall here present for consideration. One of the methods used makes it possible to study the process of evagination in the living animal, and to readily secure evaginated cysticerci without the formality of passing them into the intestine of the host.

Tænia serrata is interesting historically from the fact that it was the species first used to demonstrate the characteristic life history of a cestode. Küchenmeister took the bladderworms, known as *Cysticercus pisiformis* Zeder, from the body cavity of hares and rabbits and fed them to dogs. In the course of two to three months he found they transformed and developed into the adult *Tænia serrata*, so that proglottides were detached and lost in the fæces. When the eggs of these forms were fed to hares or rabbits, after the second day of the experiment, minute whitish cysts were discovered in the liver tissue. Subsequently, in about thirty days these parasites left the liver and developed into the full grown *Cysticercus pisiformis*. It is to be noted that this life history involves a parasitism in two entirely different hosts. In general, in the first of these hosts the young tapeworm reaches a stage in development known as a cysticercus, and usually comes to rest in some particular tissue or part of the host's body. But in order to continue development it must be

[1] Contribution No. 5 from the Zoölogy Laboratory of the Kansas State Agricultural College.

transferred to the intestine of a second or definitive host. If this transfer is not made development stops, after a time death ensues, and degeneration of the young tapeworm takes place. However, if it reaches the intestine of the definitive host, barring accidents, it evaginates the already formed scolex, attaches itself to the mucous membrane by hooks or suckers, grows to maturity, reproduces hermaphroditically, and to complete the life history all that is needed is for the eggs to reach again the particular kind of individual that may serve as an intermediate host. This is of course a matter of common knowledge.

In order to fully understand the experiment it will be best to call to mind something of the structure of a cysticercus and the transformation which takes place when it reaches the intestine. Fig. 1 is a drawing made to show the general structure of a mature cysticercus taken from a half-grown wild rabbit. It is still enclosed within the cyst. When taken from the abdominal cavity a cysticercus consists of a whitish, elongated bladder-like structure filled with a watery fluid; at the smaller end is a dense, compact mass of tissue. Examination shows that this more solid portion of the bladderworm is due to an invagination of the anterior end as shown in Fig. 3. Within the cavity of the invagination is found the apparatus, the scolex, which serves for attachment to the walls of the intestine. In this case the scolex is provided with both hooks and suckers. A few hours after a cyst has been eaten by a dog, a young tapeworm, already transformed, may be taken from the intestine. In this process the invaginated portion evaginates, the scolex is everted, and the bladder digests or is separated and lost in the fæces. Fig. 2 is a drawing made from an evaginated cysticercus that was treated with artificial digestive juices, and Fig. 4 is an enlarged drawing of the scolex of the same.

Now if *Cysticercus pisiformis* is kept in physiological saline (0.7 per cent. NaCl) it never or rarely evaginates. Fifty-five specimens were left in such a solution for three days, in fact until many had died, and at the end of that time only five had evaginated. Besides this occasional evagination, one may produce eversion of the scolex by careful physical manipulation. Braun and Lühe state that this can be readily done in the case of

Cysticercus tenuicollis. "The parasite should be held just below the head-cone in the finger and thumb of one hand, while a regular pressure from within outwards is exerted with the fingers upon the head-cone. The head-cone will lengthen and if the manipulation is repeated several times, it will turn inside out and will hang down as a flat, wrinkled, contractile band upon the surface of the bladder. If pressure is now exerted upon the free end, the head will finally become everted, springing out with a sudden jerk." I have found the process of evagination is much more difficult to produce in the smaller *Cysticercus pisiformis*, and it is not believed that the contraction of stomach or intestine of the host could have any important rôle worthy of consideration in producing the eversion of the scolex.

We must therefore look upon the process of eversion as a response to chemical rather than to physical stimuli. For when fed to a dog practically all cysticerci may evaginate under favorable circumstances. In young, previously uninfected, dogs the percentage rose as high as 90 to 100 per cent. of infection. In old dogs the percentage of infection is not so high. Whether this is due to a sort of immunity dependent upon previous infection, or to some change in the strength of the digestive juices, I am unable to say.

Since it is highly improbable that physical stimuli have any important rôle in producing eversion, it was decided to try the plan of stimulating these animals with artificial digestive juices. The following formulæ were used as a basis in preparing the solutions used in these experiments:

1. For artificial gastric juice:

Water	100.0	parts	
Hydrochloric acid	0.2	"	(dog, 0.3–0.5 part)
Pepsin	0.1	"	

2. For artificial pancreatic juice:

Water	100.0	parts	
Sodium chloride	0.6	"	
Sodium carbonate	0.2	"	(dog 0.4 part)
Pancreatin	0.2	"	

My solutions did not include all of the elements found in the natural product, but the calcium chloride and the potassium

phosphate are found in such small amounts in gastric juice that they were considered negligible. That they are not important factors in this experiment was verified by the results. For a similar reason diastase, potassium phosphate and lipase were not included in the artificial pancreatic juice which I used.

Two interesting questions had arisen in this connection: (1) Why is the young parasite not digested in the stomach? (2) What are the factors which influence or produce evagination? The answer to the first question is that as a matter of fact some of the cysticerci probably do meet the fate suggested, and that all react negatively to the gastric juice in such a way that its full effect is hindered or prevented. The answer to the second question will come out as we proceed.

In one of my experiments twenty cysticerci were removed from their cysts and ten were placed in each of two stender dishes. On these was poured a solution consisting of 0.4 per cent. of hydrochloric acid in which was dissolved a small amount of scale pepsin. Upon the addition of this liquid the bladderworms contracted strongly, especially at the invaginated end, and remained perfectly quiet, though they had previously been somewhat restless. The room temperature was about 90° Fahrenheit. Within an hour the bladders were digested enough so that they began to come apart. The cysticerci were left in this solution three and one half hours, and in all this time they showed no signs of activity, though the loose bladders were well digested. The acid solution was then removed and replaced by a solution containing the chief elements of artificial pancreatic juice as described above, leaving out the pancreatin. Within ten seconds several cysticerci had become active, and in a short time a few of them had evaginated. They were restless, however, and very soon all again drew in the scolex. Some pancreatin was now added to the solution; by the time this was dissolved most of the cysticerci were active and in the course of a few minutes ten out of twenty specimens had evaginated, though some of these had hardly completed the process. The dishes were then left over night. The next morning all were completely everted and most of them were relaxed in an excellent condition for preservation or study.

The preceding experiment indicates the probable grave danger that the parasite undergoes in passing through the dog's stomach. It also illustrates the quick response that the bladderworm gives to favorable or to unfavorable surroundings, and the response itself is a reaction to a chemical stimulus. After a considerable number of preliminary experiments in which the main facts of this paper were established, I planned a more complete series, one of which I shall give as typical of the entire set. The earlier experiments were conducted at room temperature.

First, I made up some solutions which contained the principal elements of gastric and pancreatic juices, the formulæ for which have been given. Second, several solutions were prepared containing different combinations of certain of these elements. In most cases the solution had as nearly as possible the same concentration of each component that is found in the natural digestive fluids of the dog. This is shown in the third column of Table I. Equal amounts of these solutions were next put in stender dishes and six well-developed and carefully selected cysticerci were placed in each solution. All stender dishes were then placed in a large water-bath in which the temperature varied less than one degree from 37½° C. I was unable to examine this experiment until four hours and fifty minutes later, and this allowed more time than is ordinarily required for stomach digestion. The general results of this first examination are given in the fourth column of the table. It will be noticed that in lots 2, 3 and 10, in which hydrochloric acid alone was used, there was not a single case of evagination in the eighteen specimens. The same was true for lot 1 where sodium chloride was used. The best result was produced by the artificial pancreatic juice, lot 8, in which five out of six evaginated. The next best results were found in the enzyme solutions, lots 4, 7, and 11, though the reaction to the alkaline solution of sodium carbonate might be considered just as good, lot 6. The dishes were again examined the next morning, 21 hours after the beginning. This allowed considerably longer time than that required for complete digestion, and therefore the results shown in the last column do not come out in such contrast as they do when examined somewhat earlier. For subsequent work has shown that cysticerci

when about to die will sometimes evert the scolex under unfavorable conditions.

TABLE I.

TO SHOW THE RESULTS OF AN EXPERIMENT MADE TO DETERMINE THE EFFECTS OF CERTAIN SOLUTIONS UPON THE PROCESS OF EVAGINATION. ALL MATERIAL WAS KEPT IN A WATERBATH AT ABOUT 37½° C. In lots 9, 10 and 11 the solutions were changed after four hours and fifty minutes.

Lot No.	No. Bladderworms Used.	Solution Used.	Result After 4 Hrs. 50 Min.	Result After 21 Hrs.
1	6	0.7% NaCl	None evaginated	1 evaginated
2	6	0.2% HCl	None evaginated	1 evaginated
3	6	0.4% HCl	None evaginated	1 evaginated
4	6	0.2% pepsin sol'n	1 evaginated	2 evaginated
5	6	H_2O—100 pts. HCl—0.4 pt. Pepsin—0.2 pt.	1 evaginated Bladders all digested	1 completely digested Bladder all digested
6	6	0.4 sod. Carb. sol'n	1 evaginated 1 almost evaginated	4 evaginated
7	6	H_2O—100 pts. 0.2% pancreatin	2 evaginated	4 evaginated (slightly acid)
8	6	H_2O—100 pts. NaCl—0.6 pt. Sod. carb.—0.4 pt. Pancreatin—0.2 pt.	5 evaginated	5 evaginated 1 not evaginated (young)
9	6	Sol'n lot 5 followed by Sol'n lot 6	0 evaginated	6 evaginated
10	6	Sol'n lot 3 followed by Sol'n lot 6	0 evaginated	5 evaginated 1 evaginated, partly
11	6	Sol'n lot 4 followed by Sol'n lot 7	2 evaginated	3 evaginated 1 partly evaginated

A study of the table shows the following special results: Lot 1 verifies previous observations, though the salt solution is here raised to body temperature. Therefore, we may conclude that temperature alone appears to have no important influence in producing evagination. From lots 2 and 3 it is also clear that hydrochloric acid interferes with or at least does not help eversion. The pepsin solution slightly aids the process as shown by a comparison of lots 4, 5, and 11. The pancreatin solution is a more favorable medium (lot 7). However, preceding a pancreatin solution by a pepsin solution does not seem to increase the

amount of evagination over that when it is used alone (lot 11). Lot 5 shows that artificial gastric juice is harmful to the cysticerci for it tends to digest them and undoubtedly interferes with evagination (cf. also lot 9). This last effect is due to the acid. Artificial pancreatic juice as used in lot 8 is more effective than either of its elements used separately (cf. lots 1, 6, and 7). But we find that artificial pancreatic juice produces its best results when preceded by treatment with artificial gastric juice. In lot 9 this treatment gave 100 per cent. An almost equally good result was obtained in lot 10 when treatment with an acid (hydrochloric) was followed by an alkali (sodium carbonate).

This experiment or parts of it were repeated several times, but the results were invariably similar in character. Other experiments like these were tried upon the cysticerci of *T. serialis*. The results were so much like those described that there is no need to give details here. The cysticerci of this form are more apt to be digested by the gastric juice, probably due to their smaller size. While much remains to be done in this connection, it is believed that one may safely draw the following general conclusions:

1. Treatment with artificial gastric juice followed by immersion in artificial pancreatic juice furnishes a ready and efficient means of producing the evagination of cysticerci of *T. serrata*. This is an easy method of obtaining material for preservation and for the study of the living parasites. It is best to use the gastric juice in a diluted form if one wishes to preserve the bladders intact.

2. In producing evagination the most important factors in artificial pancreatic juice are the alkali, sodium carbonate, and the extract of pancreatin. But to obtain the best reation to these stimuli previous treatment with hydrochloric acid is required.

3. Treatment with an acid followed by an alkali (lot 10) gives rather better results than treatment with pepsin followed by pancreatin (lot 11). Hence the sodium carbonate appears to be a more important factor than pancreatin in producing evagination.

4. The cysticerci are not digested in the stomach of the dog because they do not evaginate in a harmful acid medium. The

acid causes them to contract into as dense a mass as possible. This negative response to an inorganic acid is a fundamental and very general characteristic of protoplasm. For example, a very small amount of free acid produces fatal results upon protozoa, developing eggs, spermatozoa, and growing cells in general. "Sour" soils are unfavorable to the growth of most plants. Indeed, this experiment adds one more bit of evidence that one of the primary functions of the acid in the gastric juice is to kill bacteria and other living tissues that reach the stomach.

5. Finally these experiments suggest a way in which one may possibly determine why many tapeworms have a specific definitive host. Not that they have acquired a particular love for that host, but that the special host furnishes the right stimulus at the right time to call forth the proper reaction in the cysticercus. In another paper I have shown that these bladderworms will not produce tapeworms when fed to pigs, and there is one instance on record where a man swallowed five of them, without harmful results. We shall, no doubt, soon be able to explain much of this peculiar and specific behavior of parasites on the basis of a direct response to favorable or unfavorable physical or chemical stimuli. This will bring some of the little understood phenomena of parasitism into line with the brilliant work that has been done in recent years on the behavior of free-living forms.

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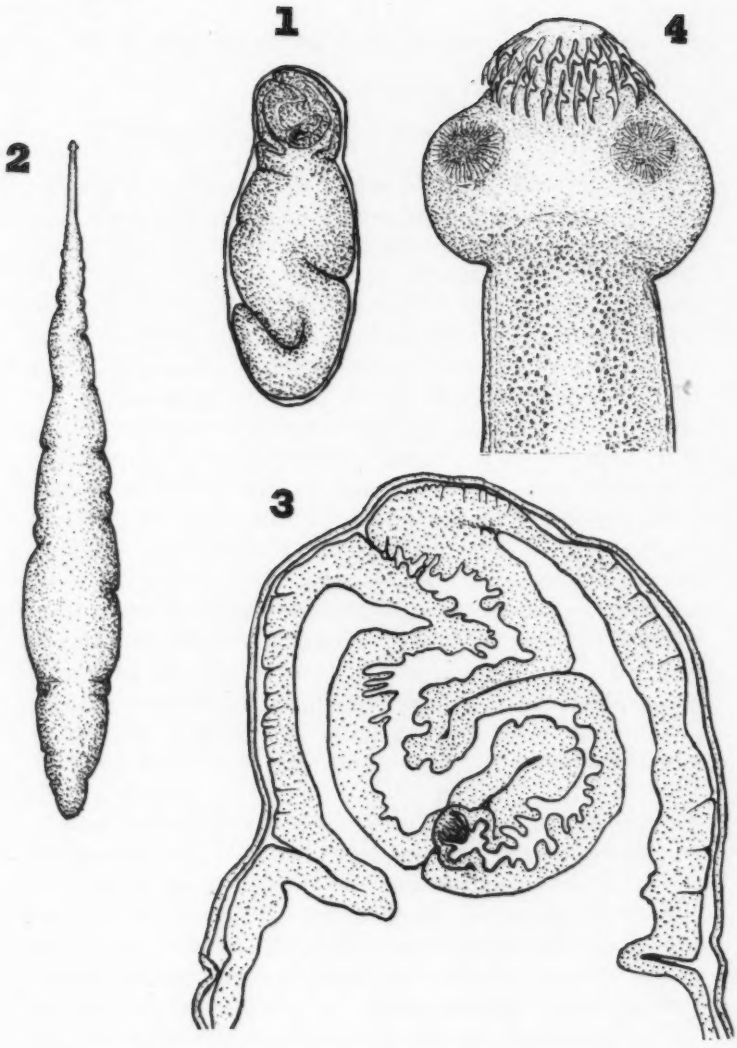
Explanation of Plate I.

Fig. 1. Diagrammatic drawing of cysticercus of *T. serrata* enclosed in cyst. (× 4.)

Fig. 2. An evaginated cysticercus of about the same size as the preceding. This specimen was treated with diluted artificial gastric juice followed by artificial pancreatic juice. (× 4.)

Fig. 3. Optical section of the anterior end of the cysticercus shown in Fig. 1, with a rather complicated invagination. Drawn with aid of camera lucida. (× 18.)

Fig. 4. Appearance of the scolex after evagination; parts slightly altered in position by pressure of cover-glass. Drawn with aid of camera lucida. (× 46.)



JOHN W. SCOTT.

ON A PECULIAR MONSTROSITY IN A FROG.

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About three years ago a student, Mr. F. L. Conover, brought me a remarkable specimen of *Rana pipiens*, which he had found among the specimens at the biological laboratory of the Madison High School. The frog was alive. It measured fifty-two millimeters body length and was apparently in full vigor, its death being caused by an overheating of my office at a time when I had to be unexpectedly absent for several days.

The remarkable character of this specimen consists in the presence of three extra limbs extending caudad from the region of the sternum, two on the right and one on the left side. It is very evident that they are attached to an extra basal piece overlying the sternum. All three are more or less imperfect, so that I find it impossible to determine whether they represent arms or legs, or both. Each has four malformed digits, and each shows a thickening indicating the location of the joint between the proximal and distal parts of the limb, the elbow or knee. All are pigmented on the surface away from the frog, with no pigment on the opposite surface. The accompanying photograph (Fig. 2) and radiograph (Fig. 1) of the specimen illustrate better than any verbal description the actual conditions present.

Monstrosities in nature involving the hind legs are not uncommon in the frog, but those involving the fore limbs are apparently rare. The only one known to me is one figured by Sutton ('92, p. 112, Fig. 60), involving the presence of an extra leg on the left side. There is a case in the human being, often quoted, shown in Fig. 3; I have been told about another closely similar case exhibited in this country several years ago, but I have not been able to get exact information concerning it.

Dr. F. R. Lillie kindly called my attention to a very important paper by Tornier (:05) which I would otherwise have overlooked.

Tornier, by an ingeniously chosen cut, separated the upper one quarter or so of the Anlagen of both hind limbs in *Pelobates*. The result was in several cases, at least, the formation of the

FIG. 1.

normal pairs of limbs *plus* two complete extra girdles each with a pair of limbs. Less completely successful experiments resulted in fewer and less fully formed supernumerary parts. But the monstrosities in all cases resembled closely those found in nature, and the close approximation and exposed situation of the Anlagen of the two hind limbs make accidents in nature which may be compared with this artificial "accident" at least not improbable. Tornier's work, it seems to me, gives us a very satisfactory explanation of such forms.

Is it equally applicable to the forelimbs? The location of the Anlagen under the opercle makes experimentation here much more difficult, and no such experiments have been carried out,

so far as I know. One might expect that by operating on one of the Anlagen a supernumerary arm, such as that of Sutton's case, could be produced. It is an experiment that some skilful operator should attempt to perform. But the Anlagen of the two limbs are here not closely approximated; they are far apart. A simultaneous operation on both is not possible, and an accident in nature equally affecting both is in the highest degree improbable.

It appears much more probable to me that in the frog here described we have a case quite parallel to that in Fig. 3, which is

FIG. 2.

properly designated, according to Schwalbe ('07), as a *Thoracopagus parasiticus*. The generally accepted, and to me very probable, explanation of these forms is that they started with a

monstrosity consisting of two complete embryos united at the breast bone. For some reason or other one gains a start in development. As their circulatory systems are united (as is believed, and in many cases demonstrated) this stronger individual (autosite) soon drives its blood into the other (parasite),

FIG. 3. (After Adami, after Wintersohn.)

the heart of which then degenerates through disuse. This means a less adequate blood supply, atrophy, and a lesser or greater absorption by the stronger individual. It is easy to see that the later in the embryonic period the inequality begins the more extensive in development will the parasite be.

I have not attempted to seek all the cases of this sort in man. Approximate parallels of that shown in Fig. 3 must be, to judge from the literature, quite rare. As to the lower vertebrates I have not been able to find a record of any case closely similar to this. It is an interesting fact to note that a frog could grow

to maturity with so very material an impediment. It makes one ask just how much of a variation from the normal is needed to enable natural selection to perform its cruel work?

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July 31, 1913.

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AN EXPLANATION OF THE NON-PRODUCTION OF FERTILIZED EGGS BY ADULT MALE-PRODUCING FEMALES IN A SPECIES OF ASPLANCHNA.

D. D. WHITNEY.

It has been observed by various investigators in the study of certain of the rotifers that in order for a male-producing parthenogenetic female to develop fertilized eggs the female must pair with the male soon after leaving the egg while she is quite young and small. No one has observed the real reason or necessity for this early pairing.

While working with a species of *Asplanchna* in the summer of 1908 at Cold Spring Harbor, New York, and again in the present summer it has been possible to watch the pairing of the two sexes under the microscope. This species is one that has very large individuals, probably as large as any species of rotifer. Both the female and the male are very transparent, thus making it possible to observe all the internal organs, including the ovaries and the eggs of the female, and the organs of the male with the living sperm in the testis. The sperm are very large and can be easily seen with the one third objective. When the male and female pair the two individuals come into contact with each other and the male keeping the head pressed against the body of the female bends the body and assuming the shape of a letter U brings the posterior end into contact also with the body of the female. Then the copulatory organ of the male is forced through the cuticle of the female into the body cavity, like a hypodermic needle, and the sperm passing through it are ejected into the body cavity of the female. The sperm immediately become active and, on account of their large size, can be easily observed swimming around in the body cavity of the female. This hypodermic injection of sperm may be made on any part of the trunk region of the female.

Many observations were made in the pairing of different young females with males. All young females observed were seen to be injected with active sperm from the male. Each female was then placed in a separate watch-glass and allowed to mature and to produce eggs or viviparous young. Some of these young females which were seen to receive living sperm into their body cavities developed parthenogenetic young daughter females and others developed fertilized thick-shelled resting eggs. It has been shown by Maupas,[1] Lauterbaum,[2] Whitney,[3] and Shull[4] that if the young male-producing female pairs with a male thick-shelled fertilized eggs will be produced, but it has never been demonstrated that in pairing the female-producing females also receive sperm into their body cavities in the same manner as do the male-producing females.

Larger and more mature parthenogenetic female-producing females as well as larger and more mature male-producing females were placed with males and many observations made. The male and female individuals come into contact with each other and the male assumes the same position as with the young females. The male makes several attempts to pierce the cuticle of the female with the piercing copulatory organ but is unsuccessful and in some instances sheds the sperm out into the water on the outside of the body of the female. These sperm are active for a few seconds, but soon become inactive and, owing probably to the injurious effects of the water, soon die. This failure of the male to inject sperm into the body cavity of both the adult female-producing female and the adult male-producing female was observed many times and in every case it was a failure. In some instances the sperm were not shed into the water by the male. This seemed to depend more or less upon the condition of the male. If it had been isolated alone for several hours and was young it would shed the sperm out into the water when in contact with the female but if it was old and partly spent it would not shed the sperm out into the water.

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This ability of the male to pierce through the cuticle of young females and the failure to pierce through the cuticle of adult females is due, probably, to the increased thickness or compactness, or both, of the cuticle of the older females. As the females grow and become older their cuticle, which is supposedly of more or less chitinous material, becomes more difficult to penetrate. It is highly probable that if the sperm could be injected into the body cavity of the adult male-producing females that the eggs would be fertilized and thick-shelled resting eggs would be produced.

Males do not seem to be able to discriminate either between each other or young females of either kind or mature females. They attempt to pair with any other individual with which they come into contact. Males have been seen to inject sperm into the body cavities of each other. Many males from a general mixed culture often contain a few free sperm actively swimming around in their body cavities. These sperm have been placed in their body cavities by other males. Thus it happens that more or less of the sperm of a male may be wasted by being injected into either young female-producing females or into other males, or even shed out into the water in attempted impossible copulation with adult females.

Summary.

1. Both kinds of young females, female-producing and male-producing, pair with male individuals and receive active sperm into their body cavities.

2. The young female-producing females having been injected with living sperm by the male mature and produce parthenogenetic daughter-females but the young male-producing females having been injected with living sperm by the male mature and produce thick-shelled fertilized eggs.

3. Male individuals pair and inject active sperm into the body cavities of each other.

4. The mature female-producing females and the mature male-producing females pair with male individuals but do not receive sperm into their body cavities because the copulatory

organ of the male is unable to pierce through the thickened cuticle of the adult female.

5. Much of the sperm of the males may be wasted by being injected into young female-producing females or into other male individuals or by being shed into the water in attempted copulation with adult females.

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